Stress, May 2013; 16(3): 311–320

ISSN 1025-3890 print/ISSN 1607-8888 online
DOI: 10.3109/10253890.2012.721824

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healthcare

Delayed memory effects after intense stress in Special Forces candidates: Exploring path processes between cortisol secretion and memory recall

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(*Received 23 January 2012; revised 29 June 2012; accepted 14 August 2012*)

Abstract
The aim of this paper is twofold. First, it explores delayed effects of high endogenously evoked cortisol concentrations on visuo-spatial declarative memory. Subsequently, it applies multiple mediation (MM) analyses to reveal path processes between stress and cognitive performance in a sample of 24 male Special Forces (SF) candidates (mean age = 27.0 years, SD = 4.1). The SF candidates were randomly assigned to a control ($n = 12$) or an intense stress group ($n = 12$), and cortisol secretion for the intense stress condition was triggered by a brusque 60 min prisoner of war exercise. Stress exposure provoked robust increases in cortisol concentrations and a significant decline in immediate recall performance, measured with the Rey–Osterrieth Complex Figure (ROCF). The relative retrieval differences in regard to the ROCF persisted even after a recovery period of 24 h, as both groups showed similar levels of memory decline over 24 h. Next, the study applied a MM design that involved distribution-independent asymptotic and resampling strategies to extend traditional bivariate analyses. MM results showed that ROCF performance was mediated by increases in cortisol concentrations. Considering the studied variables, the current analysis was the first to provide statistical support for the generally accepted thesis that cortisol secretion *in itself*, rather than subjective strain or the experimental treatment, affects cognitive performance. The revelation of such path processes is important because it establishes process identification and may refine existing paradigms.

Keywords: *Delayed recall, glucocorticoids, immediate recall, multiple mediation, real-life stress, Rey–Osterrieth Complex Figure*

Introduction

Ample studies show that increased cortisol secretion can have various immediate effects on human cognition. Tollenaar et al. (2009), however, demonstrated that a single session of exogenously administered cortisol not only has immediate negative effects, but also may result in delayed memory retrieval deficits (see also De Quervain et al. 1998). With little, if any, information about the persistence of cortisol effects on cognitive performance when evoked by endogenous reactions to naturalistic stressors, this study investigated delayed cortisol effects, nonintrusively measured in saliva, on memory retrieval after cortisol secretion was triggered by stress exposure to a strenuous Special Forces (SF) selection exercise. Empirical work combining these research features is limited and little is known about the neuroendocrinological path processes between stress exposure and memory retrieval. Yet, the identification of such processes is important because it may lead to the refinement of existing theoretical paradigms (Spencer et al. 2005; MacKinnon et al. 2007). Alternatively, as Rosenberg (1968, p. 63) neatly stated: "In the absence of such mediating or intervening mechanisms, one ends up with facts, but with incomplete understanding."

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Testing protocols were submitted to and approved by the standing ethics committee of the Open University, The Netherlands.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MAY 2013		**00-00-2013 to 00-00-2013**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Delayed memory effects after intense stress in Special Forces candidates: Exploring path processes between cortisol secretion and memory recall	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Department of Behavioral Sciences and,Epidemiology, Naval Health Research Center,San Diego,CA,85122**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Stress, May 2013; 16(3): 311?320

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**11**	

Cortisol is the principal glucocorticoid in humans and is secreted by the stress-responsive hypothalamic–pituitary–adrenal (HPA) axis, essentially a complex system of direct and indirect feedback mechanisms (Kudielka et al. 2009). Due to specific characteristics, such as its lipophilic structure and molecular size, cortisol readily passes the semi-permeable blood–brain barrier that functions as a brain-protective interface and possesses various carrier-mediated transport systems for small molecules (Ohtsuki and Terasaki 2007). Subsequently, and in interaction with other transmitter systems, cortisol can modulate memory in various ways (Joëls et al. 2006), which may depend upon the envisaged memory phase. For instance, although elevated cortisol concentrations usually facilitate memory when cortisol is released during the consolidation phase (Andreano and Cahill 2006; Smeets et al. 2008, but see Rimmele et al. 2003), it may impair memory retrieval regardless of the time of the day (De Quervain et al. 1998, 2000; Oei et al. 2007; Buchanan and Tranel 2008; Smeets 2011). The memory impairments seem to be more pronounced under higher levels of acute stress, when the activity level of the sympathetic nervous system is high (Kuhlmann and Wolf 2006; De Quervain et al. 2007). This is consistent with models that emphasize an important role for noradrenergic activity in the basolateral part of the amygdala (Roozendaal and McGaugh 2011).

Furthermore, there are indications of an inverted U-shaped relationship between cognitive performance and cortisol secretion (Andreano and Cahill 2006; Joëls et al. 2006; Salehi et al. 2010). Although moderate stress levels tend to facilitate cognitive performance, research on military populations has provided consistent evidence that intense psychological distress causes robust endocrinological alterations (Morgan et al. 2000, 2001, 2002) and reduced performance (Morgan et al. 2006; Taylor et al. 2009; Taverniers et al. 2010). McEwen and Sapolsky (1995) attributed the U-shaped relationship between cognitive performance and corticoid secretion to divergent affinities of two nuclear receptors: mineralocorticoid receptors (MRs; high affinity for cortisol) and glucocorticoid receptors (GRs; significantly lower affinity for cortisol). Memory facilitation seems to occur in the situation where MRs are fully saturated and GRs are only partially saturated with glucocorticoids. It is only when GRs are fully occupied that a decline in memory performance is observed (Abercrombie et al. 2003). Furthermore, there is some evidence of the primary role of GRs or even the MR/GR ratio (Roozendaal and McGaugh 2011), and research on both animal and human confirms the presence of dense concentrations of GRs in specific areas of the brain (Perlman et al. 2007; Patel et al. 2008). Hitherto, however, no studies provided statistical support for the above-pictured processes that represent the path processes between stress exposure and memory retrieval, respectively, via cortisol reactivity and/or subjective stress.

Tollenaar et al. (2009) investigated immediate and prolonged effects of a single dose of 35 mg cortisol on memory retrieval of emotional and neutral information. They found that exogenously administered cortisol causes significant memory impairments shortly after cortisol administration and demonstrated that this effect was not abolished after a recovery or (passive) wash-out period of 1 week. Similarly, Tollenaar et al. (2008a) found impairing effects of cortisol on long-term (6 months) memory retrieval after acute psychosocial stress (Tollenaar et al. 2008b). Tollenaar et al. (2008a) proposed that long-term memory effects could be related to diminished rehearsal and re-encoding under the influence of cortisol, thereby weakening the nonretrieved memory traces. Research in rodents, however, suggested reconsolidation—the renewed consolidation after memory traces passes a labile period during which they are prone to changes—as a possible mechanism behind delayed memory effects (e.g. Debiec et al. 2006) and that the glucocorticoid system can affect the reconsolidation mechanism of (avoidance) memory (Tronel and Alberini 2007). In human research, Hupbach et al. (2007) demonstrated reconsolidation mechanisms and labile declarative episodic memories after subtle reminders triggered integration of new information. To our knowledge, there is currently no information about the persistence of these effects on visuo-spatial declarative memory after exposure to extreme stress, except for the study of Morgan et al. (2006), which registered impaired delayed visuo-spatial memory retrieval 6 h after extreme military training. The authors introduced the Rey–Osterrieth Complex Figure (ROCF), but they focused neither on delayed effects nor on included psychoneuroendocrinological correlates.

Extending the combined work of Morgan and Tollenaar and their respective colleagues, the current field experiment examined immediate and delayed effects of cortisol reactivity under intense real-life conditions. Ongoing SF selection programs provide ideal opportunities to ethically conduct this type of research in healthy men. Given that the SF stressor neatly matched Lupien's (2009) four situational characteristics that trigger cortisol secretion (i.e. novelty, unpredictability, threat to the ego, and loss of control), excessive cortisol increases were expected for the stress group. Subsequently, the elevated cortisol concentrations were assumed to negatively affect immediate and delayed visuo-spatial recall capacities (after a 24-h recovery period; Morgan et al. 2006; Tollenaar et al. 2008a, 2008b, 2009; Taverniers et al. 2010).

The study further hypothesized that the effects of intense naturalistic stress on memory would be mediated through cortisol secretion. To investigate

this, the path processes between stress exposure and memory retrieval (direct or indirect via cortisol reactivity and/or subjective stress) were examined with a distribution-free multiple mediation (MM) procedure. A brief explanation of the applied procedure and its rationale is considered appropriate to interpret the findings (Preacher and Hayes 2008). Henceforth, it is important to note that, where "direct" of "indirect" effects are discussed, a distinction has to be made between a methodological/statistical emphasis (see further) and a neuropsychological emphasis. Importantly, the statistical emphasis reflects by no means the idea of a direct effect that cortisol would have on cognitive performance, as these are influenced by a complex interplay between diverse transmitter systems.

With regard to statistical analyses, MacKinnon (2008) argues that strictly significant two-variable relationships [e.g. bivariate correlations, *t*-tests, and analyses of variance (ANOVAs)] are a methodologically necessary, though insufficient condition to demonstrate causality. Moreover, such statistics are unable to explain how, or via which path(s), effects occur. Path identification implies the idea of mediation analysis and MM analyses are straightforward extensions of single mediator models (MacKinnon et al. 2007). MM analyses, however, contribute important additional advantages such as (1) the reduced risk of parameter bias due to omitted variables; (2) the exploration of the significance of overall indirect effects; (3) the determination of the impact of *specific* mediators—under condition of the presence of other mediators; and (4) the possibility to compare competing theories within a single research model. Given the multidimensional characteristics of stress effects, these are important advantages because, although a MM procedure arithmetically computes all variables separately, the procedure inherently acknowledges their theoretical and/or practical relatedness and this in the presence of other potentially mediating variables. Evidently, the risk of omitted variables can never be excluded, and the model can only carry out analyses according to the introduced data.

Although earlier techniques for mediation analysis were often "somewhat arcane", Preacher and Hayes (2008, p. 881) proposed a computer-intensive asymptotic and nonparametric resampling strategy (i.e. the product approach and bootstrapping) that (1) is straightforward, (2) does not require a normal data distribution and accepts skewness, and (3) admits bivariate independent variables. These characteristics render it ideal for experimental research with often small sample sizes. In Figure 1, Panel A represents a two-variable relationship between the independent variable (*X*) and the dependent variable (*Y*) via a single path (*c*). Panel B shows the MM model for indirect effects between stress (*X*) and ROCF outcomes (*Y*), via the potentially mediating variables cortisol (M_1) and subjective stress (M_2), and the respective path indices (c', a_i, b_i).

Method

The experiment was embedded within the standard annual Belgian SF selection procedure, and stress was evoked in a mock prisoner of war exercise identical to that in Taverniers et al. (2010). The precise context is restricted by confidentiality and only scientifically relevant information is provided. Testing protocols were submitted to and approved by the standing ethics committee of the Open University of the Netherlands. All procedures were carried out according to the

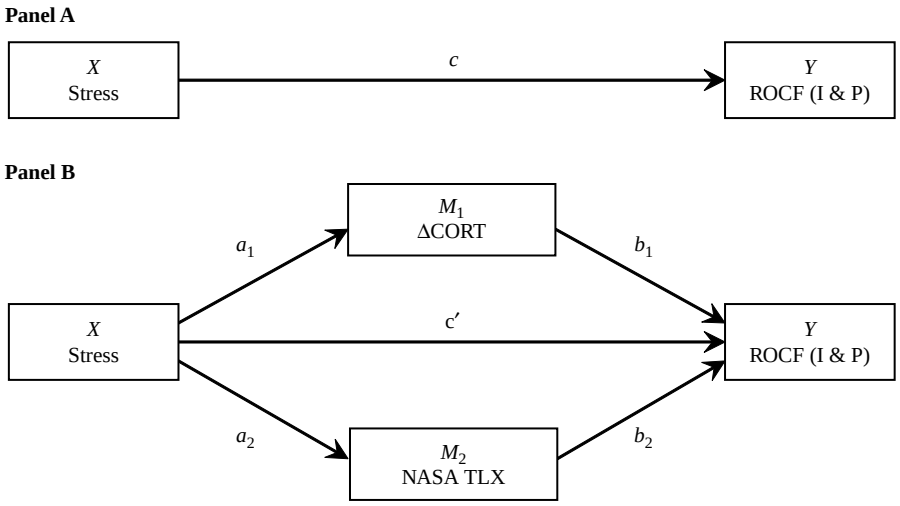

Figure 1. Panel A represents a direct effect of the independent (*X*; stress treatment) on the dependent variables [*Y*; immediate (*I*) and delayed (*P*) ROCF performance] with a single path index (*c*). Panel B represents a MM design with similar *X* and *Y*, two mediators (M_1 and M_2), and path indices (a_1, a_2, b_1, b_2, and c'). ΔCORT, change in salivary cortisol concentration; NASA TLX, National Aeronautics and Space Administration Task Load Index.

Helsinki Declaration's requirements and in full understanding, with both written and oral consent, of the participants.

Participants

Participants were 24 healthy, physically fit males with normal body mass index (Mujica-Parodi et al. 2008). Ages ranged from 21 to 35 years ($M = 27.04$ years, $SD = 4.09$). All were active duty Belgian Armed Forces members and recruitment followed as they volunteered as SF candidates. It was explained to participants that participation was voluntary and that accepting or rejecting the request to participate would by no means, positively or negatively, affect the selection result. Before the selection week, participants were medically tested, and on location assessed for endocrine disorders and the use of medication. Underscoring the strenuousness of the selection application, of the original 40 SF volunteers 16 dropped out of the selection process before the delayed visuo-spatial recall test started. Data from these candidates were not used for analyses.

Measures and materials

Saliva sampling and cortisol analyses. Salivary cortisol is a valid, reliable, and noninvasive index of unbound fractions of cortisol in the blood (Kirschbaum and Hellhammer 1989, 1994; Nicolson 2008). Salivary samples were collected with pre-numbered cotton roll devices (Salivette®; Sarstedt, Etten-Leur, The Netherlands) and stored at −20°C immediately after collection. Subsequently, the samples were thawed and centrifuged at 21.1*g* for 5 min at 4°C at the Dresdner Technical University Lab Services, Dresden, Germany. Salivary free cortisol concentrations were analyzed using a commercial chemiluminescence immunoassay (IBL, Hamburg, Germany). Mean intra- and inter-assay coefficients of variation were typically less than 8% and 12%, respectively, and the lower and upper detection limits were 0.015 μg/dl (0.41 nmol/l) and 4.0 μg/dl (110.4 nmol/l), respectively.

Subjective stress. Subjective stress was assessed by the National Aeronautics and Space Administration (NASA, Washington, DC, USA) Task Load Index (TLX; Hart and Staveland 1988), a multidimensional rating scale that combines information about the magnitude of six independent task load-related subscales (mental demands, physical demands, time demands, own performance, effort, and frustration). The TLX is considered a highly sensitive assessment technique and has often been used in military research (Rubio et al. 2004). Total scores were obtained by summing raw scores of the six subscales that ranged from 0 to 20.

Rey–Osterrieth Complex Figure. The ROCF is a standardized neuropsychological test for the evaluation of non-verbal abilities, memory recall, attention, planning, and working memory (Knight and Kaplan 2003). The complexity of the ROCF stems from 36 different elements that are difficult to memorize verbally. Although children usually apply a piecemeal approach to copy and recall, most adults use a more holistic, configurational approach. Although the traditional starting point of the test, the copy phase, essentially assesses visuo-constructive ability, the immediate recall paradigm permits the assessment of visuo-spatial abilities within declarative memory (Shin et al. 2006). The delayed recall paradigm, by contrast, allows for the assessment of delayed effects and the computation of visuo-spatial memory decline (Lezak et al. 2004). A computerized version of the ROCF was presented in black-on-white for 45 s and with a size-on-screen of 12 × 8 cm. Given the risk of a ceiling effect with healthy and highly motivated SF candidates—essentially due to the known low variability of the ROCF copy scores in healthy subjects (Shin et al. 2006), the copy phase was omitted and participants were only offered 45 seconds on-screen visual access to the ROCF. Subsequently, they had 3 min for immediate recall. After a recovery period of 24 h, the ROCF test for delayed effects was delivered in group (in a classroom), according to the unintentional, single trial, delayed recall protocol (Shin et al. 2006). For both the immediate and the delayed memory effects, the ROCFs were scored double-blind, applying the Denman scoring system (DnSS; Knight 2003). Compared to other quantitative ROCF scoring systems, the DnSS provides a larger scoring range that extends from 0 (theoretical minimum) to 72 (maximal score; 2 points per element). Higher scores represent better memory recall performance.

Control measures. The degree to which the HPA axis is activated during stressful events can show considerable individual variation depending upon character issues and life history events (Kudielka et al. 2009). Three control measures for individual differences were considered to be of interest in the current situation: (1) The 22-item impact of event scale revised (IES-R; Weiss and Marmar 1997; translated and back-translated), probably the most widely used self-report measure in the field of traumatic stress impact that assesses the potential risk of developing posttraumatic stress disorder. The IES-R was chosen for its good psychometric qualities as well as for its sensitivity to detect lower symptom levels (Creamer et al. 2003). Answering possibilities ranged from *not at all* (0) to *extremely* (4). Cronbach's α was 0.85 (Intrusions: 0.80, Hyperarousal: 0.43, and Avoidance: 0.74). (2) The 15-item

dispositional resilience scale (DRS15-R; translated and back-translated) for personality hardiness (Bartone 2007), a personality aspect that provides a natural advantage in stressful circumstances and that is associated with increased outcome performance in stress research with, among others, SF candidates (Eid and Morgan 2006; Bartone et al. 2008). Answering possibilities ranged from *not at all true* (0) to *completely true* (4). Cronbach's α was 0.60 (Commitment: 0.60, Control: 0.62, and Challenge: 0.55). (3) The generalized cognitive test battery (GCTB), a standard issued Belgian Armed Forces cognitive ability test (Irvine 2006). The GCTB assesses cognitive performances in five domains, collated to one general factor for cognitive ability that ranges from 0 to 20. No internal consistency measures are available.

Procedure. Prior to the experiment, all participants were physically and psychologically screened according to the procedures identical to those described in Taverniers et al. (2010). After arrival at the training center, candidates signed a written informed consent form, were instructed to remove all external identification marks, and received a chest number to increase anonymity. In the course of day 1, they completed the IES-R and DRS15-R scales. Participants were deprived of food, drinks, smoking, and heavy physical exercise at least 90 min prior to the cortisol measurements. They were not deprived of sleep the night before.

After a group-wise salivary cortisol baseline measurement (T_0 at 18.00 h), participants were randomly assigned to a control ($n = 12$) or stress ($n = 12$) condition, and exposed to a no-stress filler task or to SF stress treatment. The SF stress treatment consisted of a strenuous, genuinely unexpected, and uncontrollable mock prisoner of war exercise that lasted 60 ± 5 min. More specifically, participants in the stress group were abruptly and forcefully captured, physically constrained, and subsequently interrogated. To promote similar levels of cognitive load, the control group completed administration tasks and ran non-stressful weapons manipulation tasks during the same time frame.

After stress exposure, two more saliva samples were taken. Apart from the test for delayed effects, all participants were tested individually according to identical procedures. The time point for the first cortisol measurement (T_1) coincided with the start of the ROCF test (19.35 h for the first participant). The moment for the second cortisol measurement (T_2), at $T + 15$ min, was based on previous findings that indicated that a period of 15 min coincides with the highest cortisol increases (Morgan et al. 2000, 2001, 2002; Joëls et al. 2006). After running the entire exercise—more practical tests were ran after the ROCF, participants were instructed to complete the TLX, while reflecting on their respective stress exposures. Delayed cortisol effects on visuo-spatial memory were tested in group, in a class room, after a 24-h recovery period, counting from the cortisol baseline measurement. Figure 2 depicts the experimental time line and provides clock times.

Statistical analyses. When appropriate, bivariate correlations analyses, independent samples *t*-tests, and mixed model ANOVAs were used to analyze the data (SPSS 16). Peak cortisol responses at different points in time (ΔCORT) were computed as: $\Delta\text{CORT} = T_{\text{Peak}} - T_0$. If required (in case of excessive skewness), cortisol data (nmol/l) were log-transformed and Greenhouse–Geisser corrected *p*-values are reported when appropriate. Analyses were two-tailed and α was set at 0.05. Memory decline was computed as: memory decline = (immediate recall–delayed recall)/immediate recall × 100 (Lezak et al. 2004). In line with procedures described by Preacher and Hayes (2008), MM analyses (for immediate and delayed ROCF outcomes) were carried out according to the distribution of the product approach to determine the importance of the direct effect (vs. the total effect), and by

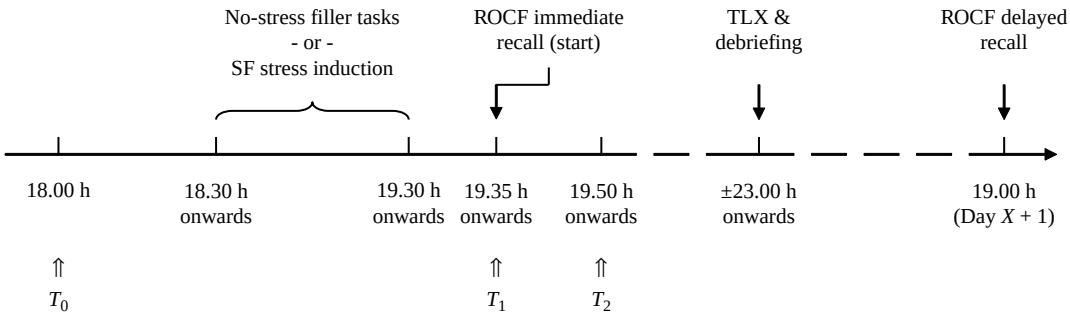

Figure 2. Experimental time line (clock times; the annotation "onwards" indicates a sequence of individual assessments with an interval of approximately 10 min) for the control versus the SF stress group; baseline saliva sampling (T_0); test instructions, cortisol saliva sampling (T_1), and subsequently ROCF immediate recall; second cortisol saliva sampling (T_2) at $T + 15$; NASA TLX scoring and debriefing; measurement of delayed effects after a 24-h recovery period. *X*, stress.

bootstrapping resampling procedures [untransformed ΔCORT data; 5000 iterations; 95% bias corrected and accelerated (BCa) confidence intervals (CI); after MacKinnon et al. (2004)] to assess both the outcome invariance of the specific mediators and the unique most significant mediator.

Results

Group equivalence

Table I shows the outcomes of all control measures and demonstrates that group equivalence was achieved.

Stress and salivary cortisol responses

Baseline salivary cortisol measurements (all subsequent cortisol measures are expressed in nmol/l) revealed no differences between the groups (two-sided independent samples *t*-test [$t(22) = 0.27$; $p = 0.79$]). The 2 (group: control and stress) × 3 (time: T_0, T_1, and T_2) mixed model ANOVA yielded a significant between subjects main effect of group [$F(1,22) = 46.12$; $p < 0.001$, partial $\eta^2 = 0.69$], a significant effect of time [Wilks' $\lambda = 0.47$, $F(2,21) = 12.03$; $p < 0.001$, partial $\eta^2 = 0.53$], and a significant group × time interaction effect [Wilks' $\lambda = 0.27$, $F(2,21) = 27.76$; $p < 0.001$, partial $\eta^2 = 0.73$]. The course of cortisol concentrations for both groups, with non-transformed data, is shown in Figure 3.

For subjective stress, an independent samples *t*-test on TLX scores after stress exposure yielded a significant difference between the control [$M = 9.17$, standard error (SE) = 1.39] and the stress ($M = 58.92$, SE = 2.80) group [$t(16.1) = -15.94$; $p < 0.001$], which qualified the circumstances as being stressful.

Immediate and delayed memory effects

A two-sided independent samples *t*-test revealed significant group differences in mean ROCF scores for the control and the stress group [$t(22) = 3.00$; $p < 0.01$], indicating that immediate ROCF recall performance had significantly deteriorated after intense stress and robust cortisol secretion. Figure 4 further shows that there were delayed stress effects as ROCF recall differences transferred over time. First, the 2 (group: control, stress) × 2 (time: $ROCF_1$, $ROCF_2$) mixed model ANOVA yielded a significant between subjects effect of group [$F(1,22) = 9.91$; $p < 0.005$, partial $\eta^2 = 0.31$], a significant effect of time [Wilks' $\lambda = 0.59$, $F(1,22) = 15.62$; $p < 0.001$, partial $\eta^2 = 0.42$], but no interaction ($p = 0.55$). In sum, the above findings indicate significant delayed effects of endogenous cortisol concentrations on memory performance after a recovery period of 24 h. Computation of memory decline determined that participants belonging to the control and the stress groups forgot at comparable rates of, respectively, 4.51% and 3.51%.

Table I. Means (*M*), standard errors of measurement (SE), and *t* values for the control measures for the control ($n = 12$) and the stress ($n = 12$) group.

	Control group		Stress group			
	M	SE	*M*	SE	*t*⋆	Significance
Age (year)	27.08	1.25	27.00	1.16	0.05	n.s.
IES-R	10.42	2.87	12.25	1.86	−0.54	n.s.
DRS15-R	36.67	0.85	35.33	0.79	1.15	n.s.
GCTB	13.73	0.72	12.85	1.11	0.66	n.s.

Note: ⋆df = 22; n.s., not significant; IES-R, Impact of event scale-revised; DRS15-R, Dispositional resilience scale 15 - revised; GCTB, Generalized cognitive test battery.

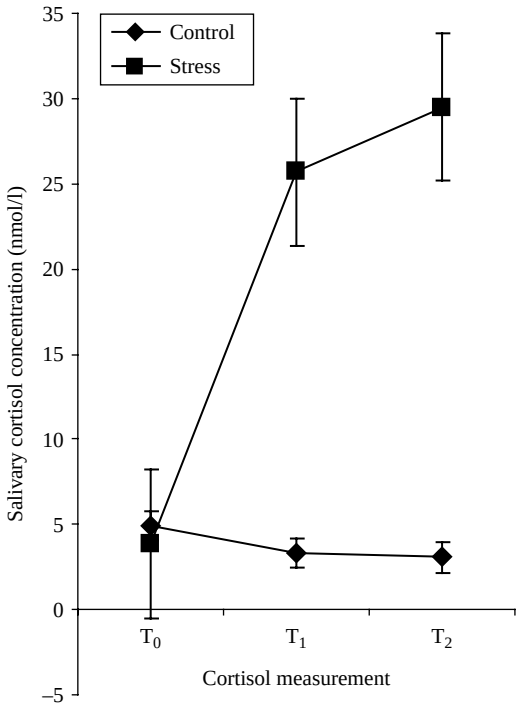

Figure 3. Result of mixed model ANOVA with untransformed salivary cortisol measures ($M \pm 1$ SE) for the control ($n = 12$) versus the SF stress group ($n = 12$) (times: T_0 at $T-75$ min, T_1 immediately poststress, and T_2 at $T + 15$ min). ⋆⋆⋆$p < 0.001$; $^{+}p = 0.07$; other differences were not significant.

Path processes

Prior to the MM analyses, bivariate correlations were calculated among all studied variables for both groups separately. For the control group, except for the two ROCF measurements [$r(12) = 0.95$, $p < 0.01$], no other significant relationships were found. Table II shows the correlations for the intense stress group. The significant correlations in the stress group provide support for the hypothesized relationship between increases in cortisol concentrations and the deterioration of both cognitive outcomes.

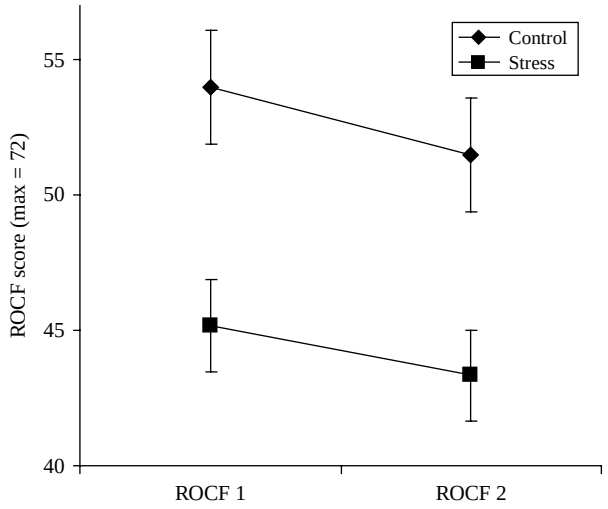

Figure 4. Result of mixed model ANOVA with pairwise comparisons, depicting mean performance scores (± 1 SE) for the control ($n = 12$) versus the SF stress group ($n = 12$) on the ROCF; immediate recall (ROCF 1) and delayed recall (ROCF 2). $^{**}p < 0.01$; $^{**}p < 0.05$.

For the immediate ROCF recall, the MM analyses revealed that the total (*c* path) and the direct effects (*c'* path) from *X* to *Y* were −8.83 ($p < 0.01$) and 1.58 (n.s.), respectively, while the directions of all *a* and *b* paths were as expected. Thus, the total indirect effect was different from zero (i.e. the combined mediators were the significant contributors to the overall effect). Examination of the specific indirect effects indicated that ΔCORT was the unique significant mediator with a BCa CI ranging from −14.06 to −2.79 (does not contain a zero; Table III). Similar results were found for the delayed ROCF as the differences between total [*c* path: −8.17, $p < 0.005$] and direct effects [*c'* path: −1.20 (n.s.)] from *X* on *Y* differed significantly. Again, all *a* and *b* path results were directed as expected. Examination of the specific indirect effects confirmed that ΔCORT was once more the unique significant mediator with a BCa CI ranging from −12.02 to −2.46 (does not contain a zero; see Table III).

Discussion

This study investigated immediate and prolonged effects of cortisol secretion that was evoked by intense naturalistic stress. First, the study confirmed that exposure to stringent military stressors triggers robust cortisol secretion, which significantly impairs immediate visuo-spatial declarative memory recall. These findings, under identical conditions, have been discussed in Taverniers et al. (2010) and accord with work under comparable stress conditions of both Morgan et al. (2000, 2001, 2002) and Taylor et al. (2007). However, by extending the study of Taverniers et al. (2010), this study demonstrated lasting effects of high endogenously evoked cortisol concentrations on visuo-spatial declarative memory.

Table II. Results from bivariate correlation analyses for the stress group ($n = 12$) concerning subjective stress (TLX), peak salivary cortisol response (ΔCORT), and both immediate and delayed ROCF scores.

	Variable	1	2	3	4
1	TLX score	–			
2	ΔCORT	0.19	–		
3	Immediate ROCF recall	−0.29	−0.77**	–	
4	Delayed ROCF recall	−0.19	−0.81**	0.91**	–

Note: $^{**}p < 0.01$.

These findings are also in line with recent studies of Tollenaar et al. (2009) who administered exogenous cortisol and employed a wash-out period of 1 week, and with those of Tollenaar et al. (2008a) who looked at memory performance after an acute laboratory stressor. The latter authors used a 6-month delay. The cognitive findings also accord with those of Morgan et al. (2006) who measured ROCF effects after a 6-h delay, but did not focus on psychoneuroendocrine correlates of visuo-spatial memory effects. Although neuropsychological work has shown more complexity (i.e. the effect of cortisol is influenced by other transmitter systems), this study introduced a sophisticated method for MM analyses (Preacher and Hayes 2008) and was, to our knowledge, the first to mathematically demonstrate the importance of cortisol reactivity as the single most significant mediator, relative to the other variables under consideration, leading to cognitive performance decline (see MacKinnon et al. 2007).

The current results also indicate that the impairing effects of cortisol on memory retrieval originate at an early stage of memory formation, as memory decay seems to remain relatively stable with similar decline rates for those who were and those who were not exposed to stress. This supports the proposed mechanism of diminished rehearsal and, more specifically, the hampered encoding and re-encoding under the influence of high cortisol concentrations.

Table III. Bootstrapping results for outcome invariance, revealing that peak cortisol response (ΔCORT), relative to the direct (total) effect and subjective stress (TLX), is the unique significant mediator between stress treatment and ROCF memory retrieval ($N = 24$).

	BCa CI		
Effect	Lower	Upper	Result
Total	−26.87| −21.44	13.89|16.18	n.s.
ΔCORT	−14.06| −12.02	−2.79| −2.46	*
TLX	−20.36| −16.22	19.55|20.46	n.s.

Notes: The table's mid-section contains the results from both the immediate and the delayed recall (immediate|delayed). n.s., not significant, *significant (95% BCa CI; 5000 iterations).

In the course of that process, non-retrieved memory traces are weakened or lost (Tollenaar et al. 2008a).

Although two-variable relationships are, strictly, a necessary though methodologically insufficient condition to determine causal relationships (Preacher and Hayes 2004, 2008; MacKinnon 2008), this study introduced MM analyses and provided mathematical support for the generally accepted idea in psychoneuroendocrinology that cortisol reactivity *in itself,* rather than the direct intense stress treatment and indirect subjective stress experiences, affects cognitive performance. The statistical revelation of a path process between stress and cognitive processing via cortisol secretion (and not via subjectively experienced stress) is novel, albeit consistent with ample findings from psychoneuroendocrinological research. As far as it concerns the studied variables, the current research replenishes related work by providing statistical support that there was a unique significant indirect effect via cortisol reactivity that caused the decline in memory performance; potentially provoked by divergent corticosteroid affinities of MRs and GRs in the brain (McEwen and Sapolsky 1995) and instigated though saturated GRs in the associated brain areas (Abercrombie et al. 2003).

Limitations

This study evidently has some limitations that need consideration to interpret its findings. First, due to the practical feasibility of inducing severe stress, the number of participants that could be recruited and tested was not high. Nevertheless, there was a significant impairing effect on memory retrieval in the stress group. Second, the study did not measure salivary cortisol concentrations at the time of the delayed ROCF test, 24 h after the stress exposure. Therefore, it cannot be determined for certain whether cortisol levels were back to baseline at that time. Given the participants' activity spectrum (identical for all), hours prior to the delayed ROCF test, one can reasonably assume that cortisol levels were significantly reduced and that the memory impairment was not due to a renewed acute stress effect. In addition to enable generalizations across sexes, populations that include female participants are desirable in the future. Accordingly, future work should envisage replications on larger and more heterogeneous population samples. Given that stress is a multifaceted phenomenon and an aggregate of a complex interplay of both subjective and objective correlates, it would be desirable to include more psychobiological measurements of stress correlates. Furthermore, it is important to note that the current MM analyses only involve the variables that were assessed in this study. Omitted variables could play an important practical role in the complex interplay between stress and cognitive performance. From a practical stance, it would be interesting in future research not only to examine the effects of diverse types of reactivation of memory traces (Hupbach et al. 2007), but also to relate memory dysfunctions to performance tasks that could range from elementary to more complex memory functions. Finally, although this study mathematically identified cortisol secretion as the principal mediator between stress and memory retrieval in two separate analyses, the latter were strongly correlated (Table II). Accordingly, further research on genuinely independent databases and, preferably, with more than two potentially mediating variables (e.g. testosterone and/or autonomic stress markers such as α-amylase) would be highly recommended to fully identify the path processes between stress and memory recall. Evidently, the MM procedure should be seen as an analytic tool and the study of such analyses would only make sense if the *a priori* defined path model conceptually makes sense.

Future directions

Despite the aforementioned limitations, this study investigated an important, though largely overlooked phenomenon, namely delayed effects of intense endogenously evoked cortisol concentrations on memory in a well-controlled real-world setting. Moreover, the study carried out MM analyses and, hitherto, no such work has been reported in the general field of psychoneuroendocrinology (MacKinnon et al. 2007). In effect, a closer look at the presently applied methodology might offer additional research opportunities. First, the current mediators were significantly correlated. MM effects, however, are often attenuated to the degree to which the mediators are correlated (comparable to the phenomenon of multicolinearity; Preacher and Hayes 2008). Given that psychobiological and subjective strain measures usually do not correlate well (Dickerson and Kemeny 2004), the applied strategy for mediation analyses might open additional possibilities for future work. Second, in (multiple) mediation analyses, a significant effect could appear even if the *a priori* relationship between the independent and the dependent variable is not significant. This situation could occur when examining opposing mediation processes and when the test of mediated effects has more statistical power than the test of the overall relation between the independent and the dependent variable (Shrout and Bolger 2002; MacKinnon 2008). Finally, Spencer et al. (2005) claim that statistical sophistication and process identification are essential for a psychological field to fully mature. From a scientific point of view and, given sufficient follow-up research, it is proposed that the demonstrated method could contribute to the refinement of existing paradigms in psychobiological stress research.

Acknowledgements

The authors would like to thank Jasper von Grumbkow for his thoughtful comments that helped to improve an earlier version of the current article.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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